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By Denholm et al. Elements are essential dietary components in human health. Enhancing nutritious element concentrations within dairy cow milk and serum, whilst ensuring concentrations of toxic elements such as heavy metals are minimized, is important both from the perspective of the health of the cow and the nutritional value of her milk for human consumption. Our results suggest element concentrations in dairy cow milk and serum are significantly influenced by both diet and genetics, and that a combination of genetic selection and dietary manipulation could be employed to alter such concentrations to improve both cow health and the healthiness of milk for human consumption.

ANALYSIS OF MILK AND SERUM ELEMENT CONCENTRATIONS

Phenotypic and genetic analysis of milk and serum element concentrations in dairy cows

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ABSTRACT

Enhancing micronutrient (*i.e.*, mineral and vitamin) concentrations within milk and serum from dairy cows is important both from the perspective of the health of the cow and the nutritive value of the milk for human consumption. However, a good understanding of the genetics underlying micronutrient content in dairy cattle is needed to facilitate such enhancements through feeding or breeding practices. In this study, milk (n=950) and serum (n=766) samples were collected from Holstein-Friesian dairy cows (n=479) on 19 occasions over a 59 month period and analyzed for concentrations of important elements. Additionally, a subset of 256 milk samples was also analyzed for concentrations of vitamin B_{12}. Cows belonged to 2 genetic lines (average and highest genetic merit for milk fat plus protein yield) and were assigned one of 2 diets based on either a by-product (BP) or home-grown (HG) ration. Univariate models accounting for repeated records were used to analyze element and vitamin B_{12} data and investigate the impact of genotype and feeding system as well as derive estimates of variance components and genetic parameters. Bivariate models were used to study correlations both within and between milk and serum. Only concentrations of Hg in milk were seen to be affected by genotype, with higher concentrations in high genetic merit cows. In contrast, element concentrations were influenced by feeding system such that cows fed the HG diet had increased milk concentrations of Ca, Cu, I, Mn, Mo, P and K, and increased serum concentrations of Cd, Cu, Fe, Mo and V. Cows on the BP diet saw increased milk concentrations of Mg, Se and Na and serum concentrations of P and Se. Heritability estimates were obtained for 6 milk and 4 serum elements, including Mg (h^2_{milk} = 0.30), Ca (h^2_{milk} = 0.20; h^2_{serum} = 0.12), Mn (h^2_{milk} = 0.14), Cu (h^2_{serum} = 0.22), Zn (h^2_{milk} = 0.24), Se (h^2_{milk} = 0.15; h^2_{serum} = 0.10) and Mo (h^2_{milk} = 0.19). Significant estimates of repeatability

were observed in all milk and serum quantity elements (Na, Mg, P, K, Ca) as well as 5 milk and 7 serum trace elements. Only K in milk and serum was found to have a significant positive genetic and phenotypic correlation (0.52 and 0.22, respectively). Significant phenotypic associations were noted between milk and serum Ca (0.17), Mo (0.19) and Na (-0.79). Additional multi-variate analyses between measures within sample type (i.e., milk or serum) revealed significant positive associations, both phenotypic and genetic, between some of the elements; in milk Se was genetically correlated with Ca (0.63), Mg (0.59), Mn (0.40), P (0.53) and Zn (0.52); whereas in serum V showed strong genetic associations with Cd (0.71), Ca (0.53), Mn (0.63), Mo (0.57), P (0.42), K (0.45) and Hg (-0.44). These results provide evidence that element concentrations in milk and blood of dairy cows are significantly influenced by both diet and genetics, and demonstrate the potential for genetic selection and dietary manipulation to alter nutrient concentration to improve both cow health and the healthiness of milk for human consumption.

Keywords: micronutrient; heavy metal; dairy cow; heritability; correlation

INTRODUCTION

Micronutrients are required throughout life and consist of vitamins and minerals that are essential for maintaining normal body function and health in humans and other animals (FAO and WHO, 2004; Gernand et al., 2016). Neither humans nor other animals can synthesize micronutrients within the body and therefore micronutrients must be obtained from the diet. When intakes of these vitamins and minerals are suboptimal this can affect normal growth and development, reducing performance as well as increasing susceptibility to, for example, Keshan disease in humans and white muscle disease in cattle due to selenium (Se) deficiency (Muth et al., 1958; Yu, 1982).

Whereas vitamins are organic molecules, minerals such as phosphorus (P), calcium (Ca), iron (Fe), zinc (Zn), Se, and iodine (I) are inorganic but are required only in very small amounts. Minerals can be further classified into trace elements (e.g. Fe, Zn, Se, I) that are required in low amounts and quantity elements (e.g. Mg, P, K, Ca) which are required in larger amounts. When intakes of quantity/trace elements or vitamins from the diet are insufficient, deficiencies can arise that can compromise animal and human health and increase the risk of disease. Indeed, there is a growing concern currently that sizable proportions of the human population do not meet micronutrient Reference Nutrient Intake (RNI) values, i.e., the amount of nutrient required to prevent deficiency (Rooke et al., 2010; Givens et al., 2014).

Dairy products, such as milk and cheese, are important sources of minerals and vitamins and contribute substantially to dietary intakes of Ca, P, I, Zn and Mg (60%, 60%, 55%, 18% and 10% of RNI, respectively) as well as vitamins A, B_2 and B_{12} (26%, 52% and 150% of RNI, respectively) (Kliem and Givens, 2011). Importantly, Mg and Ca are increasingly significant factors in bone development, especially in children (Givens et al., 2014). Furthermore, the circulating concentrations of these minerals and vitamins in the blood and milk of dairy cows likely relate to the fitness of the animal given their important roles in numerous physiological and immunological processes (Percival, 1998; Doherty, 2007; Maggini et al., 2007; Hoffmann and Berry, 2008; Prasad, 2008; Alpert, 2017). Therefore, identifying breeding strategies within the cow to increase milk micronutrient concentrations as well as optimizing micronutrient concentrations within the cow herself should be of ultimate benefit to both the cow and the human dairy product consumer. Moreover, as heavy metals such as lead (Pb), mercury (Hg), and cadmium (Cd) which have potential adverse effects on health can also be found in milk (Rey-Crespo et al., 2013), breeding strategies should also be commensurate with reducing, or at least not increasing,

concentrations of these metals where possible. Dietary manipulation of mineral concentrations in livestock has been demonstrated yet there is a relative lack of knowledge concerning the contribution of cow genetics to variation in concentrations of elements (including heavy metals) and vitamins within the blood and milk of dairy cows (Rooke et al., 2010).

The aim of this study was to carry out a phenotypic and genetic analysis of mineral, vitamin and heavy metal concentrations in dairy cow milk and serum in order to determine; 1) the effect genotype and diet on individual element and vitamin B_{12} concentrations; 2) if relationships exist between element concentrations (including vitamin B_{12}) in milk and serum; and 3) if variation between animals exists that would permit selection for optimized element and vitamin B_{12} concentrations that would be of benefit to both the health of the cow and that of the human consumer.

MATERIAL AND METHODS

Animals

Animals involved in this study were from the Langhill pedigree herd of Holstein-Friesian dairy cows (n=479) housed at the SRUC Dairy Research Centre in Dumfries, Scotland, between 2012 and 2016. All cows were part of a long-term (on-going) selection experiment for genotype x environment following a 2 by 2 approach (Veerkamp et al., 1994). Briefly, the herd has been divided equally between two distinct genetic lines (Control and Select) selected since 1970, and assigned one of two diets based on differing rations. The Control line has been bred to bulls of UK national average genetic merit for kilogram fat plus protein yield (**kg F + P**). In contrast, the high genetic merit Select line (top 5% genetic merit) has been bred from bulls with the highest genetic merit for kg F + P. The two diet groups consist of a low forage, high energy ration based on by-products and minimal land use,

simulating high-input commercial systems, and a high forage, lower energy ration based on home-grown components and using the maximum amount of land available, thus simulating low-input grazing systems (Pryce et al., 1999; Roberts and March, 2013).

The Home-grown ration (**HG**) consisted of components grown exclusively on farm and included grazed grass, grass silage, red clover silage, forage maize, lucerne silage, crimped wheat and beans. Additionally, the HG ration was balanced with purchased minerals. In contrast the By-product ration (**BP**) consisted of biscuit meal, sugar beet pulp, chopped straw, breakfast cereal, wheat distillers dark grains, soya bean meal (Hipro 50%), Vitagold, protected Fat (Megalac), molasses and minerals. Mineral compositions of both HG and BP diets are presented in Table S1 of the accompanying Supplementary Material. Target milk yields of Select cows on the low and high energy diets are 7,500 and 13,000 liters per lactation, respectively, the UK average per cow/lactation is approx. 7,557 (AHDB Dairy, 2017).

Ethics Statement

Blood sample collection was conducted in accordance with UK Home Office regulations (PPL No: 60/4278 Dairy Systems, Environment and Nutrition) and procedures were approved by the SRUC Animal Experimentation Committee. Otherwise, the study was restricted to routine on-farm observations and measurements that did not inconvenience or stress the animals.

Sampling Protocol

Samples used in the present study were collected across several years and seasons from the same on-going experimental system; 385 (of 479) cows having 2 or more samples. Furthermore, samples were selected such that they accounted for genotype and management

of cows in order to give a balanced representation of the herd. In total, 950 milk samples and 766 serum samples were collected for analysis of element and vitamin B_{12} (milk only) concentrations. Further information regarding sample collection is presented in Table S2 of the accompanying Supplementary Material.

Milk Samples. Cows in the Langhill herd are milked three times daily (AM, MD, PM) and for the present study milk samples were taken from the AM milking (at the same time as any blood sampling). Milk samples were collected on 16 separate occasions between June 2012 and January 2015 and included summer and winter periods. All milk samples were whole milk except for 256 samples which were from skimmed milk collected as part of a previous project (Denholm et al., 2017, 2018). For these latter samples, milk was first centrifuged at 3,000 × *g* for 30 mins at 4°C and the skimmed milk fraction retained from below the fat layer using a fine tipped pastette. All samples were stored at -20°C prior to analysis.

Blood Samples. Whole blood samples were collected on 13 separate occasions between April 2013 and May 2016 and included summer and winter periods. Samples were collected into plain Vacutainers (BD, Reading, UK) with blood allowed to coagulate before centrifugation at 2,000 × *g* for 10 min and the serum retained. All samples were stored at -20°C prior to analysis.

Analysis of Quantity and Trace Element and Heavy Metal Concentrations

All milk and serum samples were analyzed and concentrations of circulating quantity elements (Na, Mg, P, K, Ca), trace elements (V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, Mo, I) and heavy metals (Cd, Hg, Pb) were recorded. Milk samples (1.0ml) were digested in nitric acid (8.0ml, (65% (v/v)) using the MARS 6 digestion system (CEM, Matthews, USA) and then

stored overnight at room temperature. Samples were ramped from room temperature to 210°C and then held at this temperature for 10 min before being cooled.

Serum samples (50µl) were added to hydrogen peroxide (10µl (30% (w/w)) and nitric acid (40µl (65% (v/v)) and then digested at 85°C for 40 mins. Digested samples were diluted in decomposition matrix prior to inductively-coupled plasma mass spectrometry (ICP-MS) analysis. The decomposition matrix was nitric acid (2% (v/v)) and hydrochloric acid (0.5% (v/v)) in distilled deionized water (Millipore, UK), which was used for preparation of all solutions.

The measured isotopes analyzed by ICP-MS were ^{23}Na, ^{24}Mg, ^{31}P, ^{39}K, ^{44}Ca, ^{51}V, ^{52}Cr, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{78}Se, ^{95}Mo, ^{127}I, ^{111}Cd, ^{202}Hg, and ^{208}Pb. All element standards were used in stock solutions of 1000mg/L, which served for preparation of calibration solutions and internal standard solution. The ICP-MS measurements were carried out using the Agilent 7700X spectrometer (Agilent Technologies, UK) equipped with a MicroMist nebulizer and nickel sampler and skimmer cones. The flow of mineral standards (NIST, USA) and samples was joined together with a flow of erbium internal standard solution (1mg/L). The mixed flow (approximately 500µL /min) was delivered by the peristaltic pump to the nebulizer of the ICP-MS setup. Duration of ICP-MS analysis was 3.0 min. Data acquisition was one point, five replicates, 100 sweeps per replicate. Milk and mussel reference materials were obtained from LGC (UK).

For the quantification of Iodine in milk samples, these were first digested at 90^0C in 5% Tetramethylammonium hydroxide (TMAH) for 3h and then cooled. The TMAH (≥97%; Sigma Aldrich, Gillingham, Dorset, UK) was diluted to 5% using ultrapure water (18.2 MΩ cm, Elga PureFlex, UK). The iodine content in the milk samples was then determined by ICP-MS (7700x, Agilent Technologies, UK) in standard analysis mode using external calibration. The stock standard solution was gravimetrically prepared in-house from high

purity potassium iodide (+99.99%, Thermo Fisher Scientific, UK) in 5% TMAH. The calibration standards were prepared by serial dilution of this stock, using 5% TMAH, and a Tellurium internal standard also added at the same level as in the samples to a final concentration of 150ng/ml. The method accuracy was monitored using ERM-BD150 Skimmed Milk Powder certified reference material (LGC Standards, UK) with a certified iodine content of 1730 ± 140 µg/kg (dry weight basis).

Analysis of Vitamin B_{12}

Milk samples collected between April 2013 and January 2015 (11 sample points yielding 256 samples, n=64) were analyzed for vitamin B_{12} concentrations. Vitamin B_{12} in undiluted milk was measured using a commercial competitive assay (RIDAscreen, R-Biopharm, Germany). Absorbance was measured at 450nm where values are inversely related to vitamin B_{12} concentration using standards in the range 0 - 30µg/L. The detection limit of the assay was 0.5µg/L.

Data Preparation and Pre-Processing

Element and vitamin B_{12} data were combined with individual animal information before being subjected to quality control measures. For the purposes of the present study the interquartile range (IQR) was calculated for each trait with any concentrations falling out with $Q_1 - 1.5 \times IQR < x < Q_3 - 1.5 \times IQR$ (where Q_1 and Q_3 are the 1st and 3rd quartiles, respectively) considered an outlier and removed from the dataset. To ensure normality, all data were log-transformed prior to analysis. The final dataset consisted of 938 milk and 754 serum records, with 385 (of 479) cows having more than 1 record.

Statistical Analyses

Data were analyzed using a mixed effects linear animal model (1). Genetic relationships between individuals within the dataset were accounted for by fitting a pedigree relationship matrix. Full pedigree information spanning seven generations was available for all cows in the study.

$$\boldsymbol{y} = \boldsymbol{Xa} + \boldsymbol{Z_1 b} + \boldsymbol{Z_2 c} + \boldsymbol{e} \quad (1)$$

Here $\boldsymbol{y}$ is a vector of trait observations (i.e., mineral/vitamin/heavy metal); $\boldsymbol{a}$ is a vector of fixed effects; $\boldsymbol{b}$ is a vector of random additive genetic effects; $\boldsymbol{c}$ is a vector of permanent environmental effects; $\boldsymbol{e}$ is a vector of random residual effects; $\boldsymbol{X}$, $\boldsymbol{Z_1}$ and $\boldsymbol{Z_2}$ are incidence matrices linking phenotypic records to fixed and additive genetic, and permanent environmental effects, respectively.

Fixed effects included: diet group; genetic group; lactation number; week in milk; year × season of calving interaction; and, year × month of record interaction. Cow was fitted as a random effect to account for the additive genetic effect of the n^{th} individual cow (pedigree data for 2,793 animals was included). To account for repeated observations per cow the permanent environmental effect of the n^{th} individual cow was also fitted as a random effect. All analyses were carried out using ASReml version 3 (Gilmour et al., 2009).

RESULTS

Summary Statistics

Tables 1 and 2 summarize the element and vitamin B_{12} data generated from milk and serum samples, respectively. Trait variability was determined by calculation of the coefficient of determination (CV, %). Within milk variability was in the range 22% (Zn) to 156% (Co) and 11% (K) to 23% (Na) for trace and quantity elements, respectively. In serum, variability ranged from 24% (Se) to 133% (Mn) for trace and from 7% (Na) to 32% (P) for quantity

elements. Variability was greater in serum compared to milk and in trace elements compared to quantity elements.

Impact of Genotype and Diet on Element Concentrations

The effect of both genetic line and diet group on concentrations of micronutrients and heavy metals in milk and serum are summarized in Table 3 with *P*-values representing whether or not there was a significant difference observed between predicted mean concentrations. Analyses revealed a predisposition for increased concentrations of Hg in the milk of Select cows ($\bar{x}$ = 0.19 μg/L, *P=0.01*). No significant impact of genotype on the concentration of any other element in either milk or serum was observed. In contrast, diet was found to have a significant and varying effect on the concentration of 10 milk and 7 serum minerals. Cows on the HG diet had higher milk concentrations of Ca ($\bar{x}$ = 1257.02 mg/L, *P<0.001*), Cu ($\bar{x}$ = 61.39 μg/L, *P=0.01*), I ($\bar{x}$ = 1346.82 μg/L, *P<0.001*), K ($\bar{x}$ = 1739.23 mg/L, *P<0.001*), Mn ($\bar{x}$ = 36.82 μg/L, *P<0.001*), Mo ($\bar{x}$ = 41.89 μg/L, *P<0.001*) and P ($\bar{x}$ = 954.42 mg/L, *P<0.001*) compared to those on the BP diet (Table 3). Conversely, cows on the BP diet had higher milk concentrations of Mg ($\bar{x}$ = 116.50 mg/L, *P<0.001*), Na ($\bar{x}$ = 375.40 mg/L, *P<0.001*) and Se ($\bar{x}$ = 20.09 μg/L, *P<0.001*). Regarding serum elements, cows on the HG diet showed higher concentrations of Cd ($\bar{x}$ = 0.09 μg/L, *P<0.001*), Cu ($\bar{x}$ = 484.69 μg/L, *P<0.001*), Fe ($\bar{x}$ = 1787.19 μg/L, *P<0.02*) and Mo ($\bar{x}$ = 17.49 μg/L, *P<0.001*) in comparison to BP fed cows who showed higher serum concentrations of P ($\bar{x}$ = 154.04 mg/L, *P<0.001*), Se ($\bar{x}$ = 73.03 μg/L, *P<0.001*) and V ($\bar{x}$ = 0.53 μg/L, *P<0.001*).

Variance Components

Variance components of the milk and serum elements are presented in Tables 4 and 5, respectively. Additive genetic variance was small for both milk and serum traits and in most

cases genetic variance was higher in serum traits compared to those in milk. Heritability estimates were obtained for 17 of the 20 milk traits, 6 of which were significant (Mg, Ca, Mn, Zn, Se, I). In serum, heritability estimates were obtained for 16 of 18 traits, of which 4 were significant (K, Ca, Cu, Se). Significant element heritabilities appeared to be greater in milk traits compared to their corresponding serum trait. The highest heritability in milk and serum was observed in Mg (h^2 = 0.30, *P=0.002*) and Cu (h^2 = 0.22, *P<0.001*), respectively. Milk and serum quantity elements were all moderately to highly repeatable, and we also observed significant repeatability in 5 milk and 10 serum trace elements.

Associations Within Milk or Within Serum Elements

Correlations between element concentrations within milk are presented in Table 6 and within serum in Table 7. Strong positive genetic correlations (significantly different from zero at *P< 0.05*) were observed within milk between the quantity elements, in particular Ca, Mg and P (Table 6). Mg was positively associated with both Ca (*r* = 0.45, *P <0.001*) and P (*r* = 0.49, *P <0.001*); a positive association between P and Ca was also observed (*r* = 0.61, *P <0.001*). Moreover, strong positive genetic correlations were also observed between Se with Ca (*r* = 0.63, *P <0.001*), Mg (*r* = 0.59, *P <0.001*), Mn (*r* = 0.40, *P =0.034*), P (*r* = 0.53, *P <0.001*) and Zn (*r* = 0.52, *P <0.001*). Consistent phenotypic relationships were also observed between the milk micronutrients and are presented in full in Table 6.

Within serum, a similar set of genetic relationships were observed between the quantity elements although no significant genetic relationships were observed with Se (Table 7). The most genetically correlated nutrient in serum was V, which showed strong associations with Cd (*r* =0.71, *P =0.003*), Ca (*r* =0.53, *P <0.001*), Mn (*r* =0.63, *P* =0.*039*), Hg (*r* =-0.44, *P =0.003*), Mo (*r* =0.57, *P <0.001*), P (*r* =0.42, *P =0.006*) and K (*r* =0.45, *P*

297 *=0.006*). Phenotypically, V showed moderate to strong correlations with all other serum
298 nutrients except for Cr, Hg, Ni and Na.
299
300 ***Associations Between Milk and Serum Elements***
301 Genetic correlations of elements between milk and serum are presented in Table 8
302 with phenotypic correlations shown in Table 9 (Results from the full analysis are available in
303 Table S3 of the accompanying Supplementary Material). Significant additive genetic
304 correlations (significantly different from zero at $P<0.05$) were found to exist between a
305 number of the milk and serum elements with most being positive (Table 8). The strongest
306 negative associations were observed between serum Ni with milk V (r = -0.98, P = 0.008),
307 Co (r = -0.86, P = 0.011) and Na (r = -0.62, P = 0.017). Potassium (K) was the only element
308 that was found to have a significant correlation between concentrations recorded in milk and
309 serum (r = 0.45, P = 0.025). Further, milk K was found to be significantly positively
310 correlated with serum Mg (r = 0.53, P = 0.008), Co (r = 0.48, P = 0.039), Mo (r = 0.45, P =
311 0.020), and Cd (r = 0.43, P = 0.035). Moreover, serum Mg was highly correlated with milk
312 Ca (r = 0.54, P = 0.014), Mn (r = 0.57, P = 0.028) and P (r = 0.49, P = 0.029). Associations
313 involving heavy metals were only observed between serum Cd and milk Se, K and Mn.
314 All phenotypic correlations obtained between milk and serum element concentrations
315 are presented in Table 9. Statistically significant correlations were obtained for Ca (r = 0.17,
316 P = 0.019), Mo (r = 0.19, P = 0.009), K (r = 0.19, P = 0.006) and Na (r = - 0.79, P <
317 0.001). Serum Na was also found to be strongly positively correlated with milk Ca (r = 0.81,
318 P < 0.001), Zn (r = 0.74, P < 0.001), K (r = 0.64, P < 0.001) and Mg (r = 0.49, P = 0.024).
319 The majority of associations observed were positive but negative correlations were noted
320 between milk Cr and serum Se (r = -0.16, P = 0.029); milk Fe with serum Pb (r = -0.62, P <
321 0.001) and serum Cd (r = -0.288, P = 0.002); milk Hg with serum Cu (r = -0.23, P = 0.003)

and serum Mo (r = -0.20, P = 0.034); milk Zn with serum Co (r = -0.15, P = 0.033); and milk B_{12} with serum Ni (r = -0.38, P < 0.001) (Table 9). It was noted that Cd and Pb in milk as well as Cr in serum showed no associations with any other nutrient whether in milk or serum.

DISCUSSION

The main aim of this study was to estimate (co)variance components of important dairy cattle milk and serum elements (minerals, heavy metals), as well as milk vitamins B_{12}, in order to explore potential selection strategies for optimizing concentrations both for the benefit the cow and the human dairy product consumer. Significant heritability estimates were obtained for 6 milk and 4 serum minerals in addition to repeatability estimates for 10 milk and 15 serum elements (Tables 4 and 5). From the literature, the majority of genetic analyses previously reported correspond to quantity elements in milk (a summary of h^2 values found in the literature can be found in Table S4 of the accompanying Supplementary Material). Milk Ca, Mg, P, K and Na have been shown to have heritabilities ranging from 0.10 (Toffanin et al., 2015) to 0.72 (Buitenhuis et al., 2015), 0.08 (Buitenhuis et al., 2015) to 0.60 (van Hulzen et al., 2009), 0.12 (Toffanin et al., 2015) to 0.62 (van Hulzen et al., 2009), 0.19 (Visentin et al., 2018) to 0.46 (van Hulzen et al., 2009), and 0.20 (Buitenhuis et al., 2015) to 0.24 (Visentin et al., 2018), respectively. Heritability estimates for some milk trace elements have also been reported including Cu (0.28, Buitenhuis et al., 2015), Fe (0.15, Buitenhuis et al., 2015), Mn (0.13, Buitenhuis et al., 2015), Se (0.20, van Hulzen et al., 2009; 0.20, Buitenhuis et al., 2015) and Zn (0.41, van Hulzen et al., 2009; 0.57, Buitenhuis et al., 2015). Regarding serum, a genetic analysis of Ca, Mg, P and K carried out by Tsiamadis et al. (2016) reported heritabilities of 0.20, 0.21, 0.25 and 0.10, respectively. Furthermore, heritabilities of serum Cu and Zn have both been reported as 0.22 (Morris et al., 2006). The

results from the present study are within these ranges for these nutrients and we also investigated a number of milk and serum elements (including heavy metals) that we believe have not yet been reported in dairy cows. As such, we believe this is the first study to estimate heritability of the milk trace element Mo (0.19) as well as repeatability estimates for milk and serum trace elements and heavy metals.

Concentrations of I in milk are known to be affected by a number of different factors including dietary iodine level and the presence of iodine antagonists, such as glucosinolates, in the feed, farm management practices, teat dipping with iodine-containing substances, and milk processing (Flachowsky et al., 2014). In the present study milk I was influenced by diet type and was significantly repeatable (0.24, $P=0.005$), this should be important given the importance on milk and dairy products to UK intakes of iodine (Kliem and Givens, 2011). Although mean milk I concentrations were much higher than those listed in the current UK Food Composition Database (Food Standards Agency, 2015), it is important to note that the current study analyzed raw milk and that pasteurization is known to substantially reduce I concentrations in milk (Nazeri et al., 2015).

Sodium is another essential quantity element which has been shown to be an important factor in milk production (Derrig et al., 1974; Spek et al., 2013) and is lost though milk, urine, saliva and faeces (Renkema et al., 1962). We observed a strong negative phenotypic association between concentrations of Na in milk and serum (r=-0.79, $P<0.001$) suggesting that increased milk Na concentrations correspond to a decrease in serum concentrations. During lactation Na concentrations of milk have been shown to increase (Gueguen et al., 1961; Safwate et al., 1981) whereas in blood large variations (dependent on physiological condition or age) have been observed (Skrzypczak et al., 2013). Moreover, it has been hypothesized that decreased Na concentrations in early lactation may be due to decreased plasma rennin activity post calving (Ożgo et al., 2008).

372 Milk is also an excellent source of vitamin B_{12} and milk and dairy products contribute
373 significantly to vitamin B_{12} intakes in humans (150% of RNI, Henderson et al., 2003a; b;
374 Kliem and Givens, 2011) making it an attractive breeding target in terms of enhancing
375 nutrient quality for the consumer. Vitamin B_{12} contains Co and Co is required in the diet of
376 cattle in order that this vitamin is synthesized endogenously by rumen bacteria (Stemme et
377 al., 2008). Although the concentration of Co in serum was repeatable (0.14, P=0.019), we did
378 not observe significant repeatability in milk Co or vitamin B_{12}. The estimated heritability for
379 vitamin B_{12} in milk was not significant (h^2=0.12, P=0.18) and this was also true for milk and
380 serum Co (h^2=0.04, P=0.31; h^2=0.07, P=0.30, respectively). Furthermore, we found no
381 significant associations between milk vitamin B_{12} and Co in either milk or serum.
382 Genetic line (average or highest genetic merit for milk fat plus protein yield) had no
383 significant effect on circulating element or vitamin B_{12} concentrations in either milk or blood
384 serum with the exception of the heavy metal Hg which showed higher concentrations in the
385 milk of Select line cows. Moreover, due to cows being part of an experimental research herd
386 any biases in management between the genetic lines were non-existent such that cows within
387 the same line but on diverging diets were consequently unaffected by management decisions
388 (Pryce et al., 1999). Concentrations of elements in both milk and serum were effected
389 depending on whether the cow was fed the Home-grown or By-product ration. This has
390 potential benefits for manipulation of nutrient content through changes in management alone,
391 a benefit that could be complemented/improved through selection and breeding. It also
392 suggests that selection for higher milk fat and protein is independent of blood or milk
393 micronutrient concentrations.
394 Given the mostly positive genetic correlations among the milk minerals examined in
395 the present study, selection alone for one milk mineral might be expected to also increase the
396 concentrations of other minerals. Therefore, selection for milk Ca would likely boost P, Zn

and Se concentrations for example, leading to multiple improvements in milk mineral concentrations for the benefit of the human dairy consumer.

Furthermore, since heavy metals have adverse effects on health, any breeding objectives should also be directed towards minimizing concentrations of these metals or at least, not to increase concentrations. The findings of this study identified few significant genetic associations of heavy metals with micronutrient concentrations and, in cases where a significant association was found, these tended to be negative. This suggests that genetic selection programs aimed at increasing micronutrient concentrations should not inadvertently increase concentrations of toxic heavy metals. The minimum risk level (MRL) has not been established for Cd or Hg in milk, but the MRL for Pb in EU milk is 20 $\mu g\,kg^{-1}$ (CE Regulation no. 2001/466). The Pb concentrations as found in milk in this study were below levels of food safety concern in the EU.

It is interesting to note that while significant phenotypic relationships were observed between some milk and corresponding serum element measurements, only one genetic association was identified (between milk and serum K). Moreover, we observed stronger and additional relationships between differing nutrients between milk and serum. Results from the present study agree with those of Wang et al. (2014) in that concentrations of Cu, Fe and Zn in milk do not reflect corresponding serum concentrations. Additionally, our findings suggest that the same is true of all elements examined in the present study with the exception of Na, K, Ca, and Mo.

CONCLUSIONS

Through the present study we have established that circulating concentrations of elements in both the milk and serum of dairy cows are significantly influenced by genetics and feeding system. As expected, diet had a significant effect on mineral concentrations,

especially in milk, and as such provided a potential route for manipulation via changes in rations. The results presented provide clear evidence that many of such traits are heritable indicating that selection for desired element concentrations in both milk and serum is possible. This work will help inform industry solutions to better improve both genetics and management practices for the benefit of not only the cow but also the healthiness of the milk for the consumer.

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552 **Table 1.** Descriptive statistics of the milk element and vitamin B_{12} data

Nutrient	Count	Cows[1]	Mean	SD	Minimum	Maximum	CV%†
Sodium (Na, mg/L) [q]	878	202/318	364.16	83.97	148.92	602.97	23.06
Magnesium (Mg, mg/L) [q]	908	206/320	113.19	15.58	69.74	155.98	13.76
Phosphorus (P, mg/L) [q]	904	205/318	946.18	135.04	586.19	1,300.76	14.27
Potassium (K, mg/L) [q]	892	202/317	1,774.39	191.81	1,239.38	2,301.43	10.81
Calcium (Ca, mg/L) [q]	900	203/318	1,192.18	181.14	679.46	1,684.86	15.19
Vanadium (V, µg/L) [t]	186	103/184	2.70	1.96	0.06	7.22	72.43
Chromium (Cr, µg/L) [t]	726	188/298	25.54	20.14	0.29	104.68	78.87
Manganese (Mn, µg/L) [t]	889	204/320	38.08	14.49	1.06	83.46	38.05
Iron (Fe, µg/L) [t]	848	194/312	1,077.02	886.26	41.22	4,017.03	82.29
Cobalt (Co, µg/L) [t]	411	80/164	4.30	6.71	0.06	30.40	156.12
Nickel (Ni, µg/L) [t]	321	115/232	427.28	463.02	0.86	2,113.01	108.37
Copper (Cu, µg/L) [t]	916	210/324	120.77	78.20	0.96	386.82	64.75
Zinc (Zn, µg/L) [t]	919	208/321	4,348.23	938.97	1,916.91	6,833.17	21.59
Selenium (Se, µg/L) [t]	916	208/323	19.10	4.67	6.83	31.81	24.46
Molybdenum (Mo, µg/L) [t]	888	200/314	37.20	12.14	6.26	71.86	32.63
Iodine (I, µg/L) [t]	448	131/267	1,448.35	610.52	161.20	3,290.90	42.15
Cadmium (Cd, µg/L) [h]	733	205/311	0.16	0.10	0.01	0.45	61.80
Mercury (Hg, µg/L) [h]	417	188/301	0.31	0.26	0.01	1.04	83.35
Lead (Pb, µg/L) [h]	829	203/316	4.82	3.17	0.02	15.72	65.75
Vitamin B_{12} (B_{12}, µg/L)	247	63/64	0.82	0.38	0.50	1.75	47.08

553 1 Number of cows (/ total cows) with at least 2 observations of trait
554 † Coefficient of variation
555 q Quantity element
556 t Trace element
557 h Heavy metal
558

559

560 **Table 2.** Descriptive statistics of the serum element data

Nutrient	Count	Cows[1]	Mean	SD	Minimum	Maximum	CV%†
Sodium (Na, mg/L) [q]	169	1/168	3,133.18	210.64	2,533.07	3,757.49	6.72
Magnesium (Mg, mg/L) [q]	734	215/322	23.01	5.11	9.79	36.62	22.22
Phosphorus (P, mg/L) [q]	743	214/323	139.07	44.97	20.75	260.98	32.33
Potassium (K, mg/L) [q]	732	215/323	206.02	35.54	105.38	302.45	17.25
Calcium (Ca, mg/L) [q]	707	214/318	105.37	17.76	58.03	153.36	16.86
Vanadium (V, µg/L) [t]	584	213/214	0.68	0.21	0.15	1.25	31.15
Chromium (Cr, µg/L) [t]	221	63/214	0.89	0.76	0.00	4.23	85.71
Manganese (Mn, µg/L) [t]	626	215/259	6.46	8.61	0.07	36.70	133.22
Iron (Fe, µg/L) [t]	654	213/263	2,179.44	1,144.67	292.15	6,157.91	52.52
Cobalt (Co, µg/L) [t]	610	214/230	1.34	0.68	0.11	4.39	50.73
Nickel (Ni, µg/L) [t]	392	201/212	3.85	2.71	0.01	12.14	70.31
Copper (Cu, µg/L) [t]	726	215/315	601.02	195.51	108.52	1,113.46	32.53
Zinc (Zn, µg/L) [t]	626	214/258	910.90	330.06	201.57	2,109.51	36.23
Selenium (Se, µg/L) [t]	717	214/322	75.96	18.58	26.11	124.59	24.45
Molybdenum (Mo, µg/L) [t]	662	165/321	14.92	13.62	1.26	62.88	91.30
Cadmium (Cd, µg/L) [h]	666	215/283	0.11	0.11	0.00	0.46	100.76
Mercury (Hg, µg/L) [h]	265	139/151	3.43	3.22	0.00	14.89	93.97
Lead (Pb, µg/L) [h]	738	215/325	83.23	81.74	0.01	367.56	98.20

561 1 Number of cows (/ total cows) with at least 2 observations of trait
562 † Coefficient of variation
563 q Quantity element
564 t Trace element
565 h Heavy metal
566

Table 3. Impact of diet and genotype on element concentrations in milk and serum. Predicted mean values for the By-product and Home-grown diets, as well as for Control and Select genetic lines, obtained via univariate models (accounting for all other sources of systematic variation). Only predicted mean concentrations that were significantly different (*P<0.05*) are presented

Nutrient	$\bar{x}_{BP}$	$\bar{x}_{HG}$	SED‡	*P*
Milk				
Sodium (Na, mg/L) [q]	375.40	338.32	1.02	*<0.001*
Magnesium (Mg, mg/L) [q]	116.50	110.81	1.01	*<0.001*
Phosphorus (P, mg/L) [q]	878.40	954.42	1.01	*<0.001*
Potassium (K, mg/L) [q]	1,661.21	1,739.23	1.01	*<0.001*
Calcium (Ca, mg/L) [q]	1,107.32	1,257.02	1.01	*<0.001*
Manganese (Mn, µg/L) [t]	27.49	36.81	1.03	*<0.001*
Copper (Cu, µg/L) [t]	54.55	61.39	1.05	0.009
Selenium (Se, µg/L) [t]	20.09	15.14	1.01	*<0.001*
Molybdenum (Mo, µg/L) [t]	32.90	41.89	1.02	*<0.001*
Iodine (I, µg/L) [t]	1,082.79	1,346.82	1.04	*<0.001*
Serum				
Phosphorus (P, mg/L) [q]	154.04	108.80	1.02	*<0.001*
Vanadium (V, µg/L) [t]	0.53	0.42	1.03	*<0.001*
Iron (Fe, µg/L) [t]	1,663.20	1,787.19	1.03	0.019
Copper (Cu, µg/L) [t]	418.01	484.69	1.02	*<0.001*
Selenium (Se, µg/L) [t]	73.02	64.65	1.02	*<0.001*
Molybdenum (Mo, µg/L) [t]	5.46	17.49	1.05	*<0.001*
Cadmium (Cd, µg/L) [h]	0.04	0.09	1.09	*<0.001*
	$\bar{x}_{C}$	$\bar{x}_{S}$	SED‡	*P*
Milk				
Mercury (Hg, mg/L) [h]	0.14	0.19	1.11	0.010

$\bar{X}_{BP}$ Predicted By-product mean
$\bar{x}_{HG}$ predicted Home-grown mean
$\bar{X}_{C}$ Predicted Control line mean
$\bar{X}_{S}$ Predicted Select line mean
‡ Standard error difference
q Quantity element
t Trace element
h Heavy metal

Table 4. Variance Components and heritability (h^2) estimates of the milk elements and vitamin B_{12} data

Nutrient	σ_a	σ_{pe}	σ_p	h^2 (S.E.)	*Repeatability*
Sodium (Na, mg/L) [q]	0.000	0.006	0.039	N.E.	0.16* (0.040)
Magnesium (Mg, mg/L) [q]	0.005	0.000	0.016	0.30* (0.090)	0.30* (0.044)
Phosphorus (P, mg/L) [q]	0.002	0.002	0.016	0.12 (0.070)	0.22* (0.042)
Potassium (K, mg/L) [q]	0.001	0.002	0.010	0.11 (0.078)	0.27* (0.045)
Calcium (Ca, mg/L) [q]	0.003	0.000	0.015	0.20* (0.078)	0.22* (0.043)
Vanadium (V, µg/L) [t]	0.063	0.000	0.978	0.06 (0.161)	0.06 (0.161)
Chromium (Cr, µg/L) [t]	0.013	0.000	0.450	0.03 (0.035)	0.03 (0.035)
Manganese (Mn, µg/L) [t]	0.020	0.000	0.139	0.14* (0.039)	0.14* (0.039)
Iron (Fe, µg/L) [t]	0.006	0.000	0.531	0.01 (0.028)	0.01 (0.028)
Cobalt (Co, µg/L) [t]	0.039	0.000	0.963	0.04 (0.056)	0.04 (0.056)
Nickel (Ni, µg/L) [t]	0.049	0.000	1.247	0.04 (0.093)	0.04 (0.093)
Copper (Cu, µg/L) [t]	0.018	0.000	0.413	0.04 (0.028)	0.04 (0.028)
Zinc (Zn, µg/L) [t]	0.011	0.009	0.047	0.24* (0.116)	0.43* (0.042)
Selenium (Se, µg/L) [t]	0.005	0.001	0.033	0.15* (0.072)	0.18* (0.041)
Molybdenum (Mo, µg/L) [t]	0.011	0.000	0.060	0.19* (0.041)	0.19* (0.041)
Iodine (I, µg/L) [t]	0.031	0.003	0.140	0.22 (0.123)	0.24* (0.081)
Cadmium (Cd, µg/L) [h]	0.000	0.000	0.511	N.E.	N.E.
Lead (Pb, µg/L) [h]	0.000	0.000	0.577	N.E.	N.E.
Mercury (Hg, µg/L) [h]	0.038	0.000	1.009	0.04 (0.064)	0.04 (0.064)
Vitamin B_{12} (B_{12}, µg/L)	0.034	0.008	0.346	0.10 (0.226)	0.12 (0.095)

σ_a Additive genetic SD

σ_{pe} Permanent environment SD

σ_p Total phenotypic SD

q Quantity element
t Trace element
h Heavy metal
* Significantly different from zero at $P<0.05$
N.E. Not estimable due to additive genetic variance → 0

Table 5. Variance Components and heritability (h^2) estimates of the serum elements data

Nutrient	σ_a	σ_{pe}	σ_p	h^2 (S.E.)	*Repeatability*
Sodium (Na, mg/L) [q]	0.001	0.003	0.004	0.34 (0.234)	1.00* (0.002)
Magnesium (Mg, mg/L) [q]	0.007	0.000	0.047	0.14 (0.083)	0.14* (0.050)
Phosphorus (P, mg/L) [q]	0.006	0.007	0.062	0.09 (0.079)	0.20* (0.046)
Potassium (K, mg/L) [q]	0.004	0.000	0.024	0.18* (0.051)	0.18* (0.051)
Calcium (Ca, mg/L) [q]	0.003	0.000	0.025	0.12* (0.049)	0.12* (0.049)
Vanadium (V, µg/L) [t]	0.007	0.017	0.073	0.09 (0.121)	0.33* (0.059)
Chromium (Cr, µg/L) [t]	0.000	0.000	1.215	N.E.	N.E.
Manganese (Mn, µg/L) [t]	0.012	0.000	0.324	0.04 (0.044)	0.04 (0.044)
Iron (Fe, µg/L) [t]	0.012	0.000	0.130	0.09 (0.046)	0.09 (0.046)
Cobalt (Co, µg/L) [t]	0.009	0.007	0.124	0.07 (0.100)	0.14* (0.055)
Nickel (Ni, µg/L) [t]	0.245	0.113	0.830	0.30 (0.183)	0.43* (0.076)
Copper (Cu, µg/L) [t]	0.018	0.000	0.084	0.22* (0.051)	0.22* (0.051)
Zinc (Zn, µg/L) [t]	0.007	0.005	0.067	0.11 (0.095)	0.18* (0.054)
Selenium (Se, µg/L) [t]	0.005	0.000	0.053	0.10* (0.047)	0.10* (0.047)
Molybdenum (Mo, µg/L) [t]	0.000	0.093	0.335	N.E.	0.28* (0.057)
Cadmium (Cd, µg/L) [h]	0.044	0.182	0.909	0.05 (0.086)	0.25* (0.059)
Lead (Pb, µg/L) [h]	0.062	0.153	0.546	0.11 (0.103)	0.39* (0.051)
Mercury (Hg, µg/L) [h]	0.709	0.613	1.433	0.49 (0.347)	0.92* (0.017)

σ_a Additive genetic SD

σ_{pe} Permanent environment SD

σ_p Total phenotypic SD

q Quantity element
t Trace element
h Heavy metal
* Significantly different from zero at $P<0.05$
N.E. Not estimable due to additive genetic variance → 0

Table 6. Correlations between element concentrations and vitamin B_{12} within milk. Additive genetic correlations are presented above the diagonal with phenotypic below. Corresponding standard errors are given in parenthesis

	Na	Mg	P	K	Ca	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Se	Mo	I	Hg	B_{12}
Na		0.16	-0.13	-0.26	0.03	0.56	-0.60	-0.04	-0.02	0.32		-0.27	-0.15	0.09	-0.06	-0.21	0.78	0.17
		(0.16)	(0.21)	(0.20)	(0.19)	(0.88)	(0.47)	(0.22)	(0.58)	(0.55)	-	(0.36)	(0.16)	(0.20)	(0.19)	(0.24)	(0.63)	(0.40)
Mg	0.36*		0.49*	0.20	0.45*	-0.47	-0.84	0.20	0.05	-0.12	0.56	-0.24	0.17	0.59*	0.13	0.10	-0.01	-0.05
	(0.04)		(0.11)	(0.13)	(0.12)	(0.91)	(0.59)	(0.17)	(0.45)	(0.38)	(0.85)	(0.26)	(0.11)	(0.11)	(0.15)	(0.19)	(0.44)	(0.31)
P	0.39*	0.56*		-0.04	0.61*	-0.61	-0.11	0.55*	0.25	0.31		0.31	0.23	0.53*	0.22	-0.27	-0.07	0.21
	(0.04)	(0.03)		(0.17)	(0.09)	(1.28)	(0.37)	(0.16)	(0.53)	(0.44)	-	(0.30)	(0.12)	(0.13)	(0.16)	(0.20)	(0.50)	(0.34)
K	0.19*	0.35*	0.54*		0.02	-0.57	0.27	0.29	0.31	0.46	0.15	-0.19	-0.05	-0.09	0.02	0.12	0.13	0.18
	(0.04)	(0.04)	(0.03)		(0.16)	(0.75)	(0.38)	(0.17)	(0.58)	(0.47)	(0.53)	(0.27)	(0.13)	(0.17)	(0.16)	(0.20)	(0.49)	(0.37)
Ca	0.30*	0.57*	0.75*	0.43*		-0.29	0.01	0.57*	-0.07	-0.15	0.93	0.37	0.25*	0.63*	0.14	-0.05	0.22	0.15
	(0.04)	(0.03)	(0.02)	(0.04)		(0.79)	(0.39)	(0.17)	(0.54)	(0.43)	(0.89)	(0.29)	(0.12)	(0.12)	(0.16)	(0.21)	(0.70)	(0.36)
V	-0.19	0.01	-0.23*	-0.07	0.02		0.70	0.72	0.84	0.42			-0.08	0.32	-0.29	0.46	0.36	
	(0.10)	(0.10)	(0.10)	(0.11)	(0.11)		(1.44)	(1.10)	(2.11)	(1.42)	-	-	(0.52)	(0.80)	(0.97)	(1.04)	(1.37)	-
Cr	0.07	-0.02	0.01	0.02	-0.05	0.02		-0.04	-0.62	-0.76			-0.16	-0.62	0.03	-0.85	0.07	
	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)	(0.11)		(0.40)	(1.65)	(1.55)	-	-	(0.30)	(0.44)	(0.35)	(0.70)	(1.03)	-
Mn	0.19*	0.17*	0.22*	0.08	0.16*	-0.08	0.21*		0.01	0.13	0.55	0.24	0.41*	0.40*	0.26	-0.02	-0.42	0.33
	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)	(0.09)	(0.04)		(0.69)	(0.37)	(0.52)	(0.31)	(0.14)	(0.18)	(0.18)	(0.24)	(0.53)	(0.40)
Fe	0.10*	0.04	0.07	0.05	-0.02	-0.06	0.40*	0.42*			0.83	0.66	0.05	0.24	0.30	-0.34		
	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)	(0.10)	(0.04)	(0.03)		-	(1.04)	(1.05)	(0.43)	(0.50)	(0.59)	(0.74)	-	-
Co	0.07	0.11	0.14*	0.04	-0.04	-0.00	0.32*	0.37*	0.59*			-0.42	0.30	0.26	-0.76	-0.44		-0.82
	(0.06)	(0.06)	(0.06)	(0.06)	(0.06)	(0.12)	(0.06)	(0.05)	(0.03)		-	(0.69)	(0.51)	(0.40)	(1.14)	(0.57)	-	(1.19)
Ni	-0.23*	-0.11	0.04	-0.00	0.10		0.09	0.17*	0.50*				0.01	-0.05	0.38		0.99	
	(0.07)	(0.07)	(0.07)	(0.07)	(0.07)	-	(0.07)	(0.07)	(0.05)	-		-	(0.39)	(0.43)	(0.89)	-	(2.42)	-
Cu	0.04	0.02	0.01	-0.09*	0.04	0.09		0.03	-0.09*	-0.24*			0.20	0.27	-0.21	-0.64	0.19	
	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)	(0.10)	-	(0.04)	(0.04)	(0.05)	-		(0.24)	(0.29)	(0.29)	(0.35)	(0.78)	-
Zn	0.22*	0.37*	0.38*	0.15*	0.35*	0.00	0.13*	0.25*	0.15*	0.22*	-0.13	0.11*		0.52*	0.16	-0.04	-0.28	0.28
	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)	(0.10)	(0.04)	(0.04)	(0.04)	(0.06)	(0.07)	(0.04)		(0.10)	(0.13)	(0.17)	(0.48)	(0.30)
Se	0.32*	0.49*	0.43*	0.15*	0.42*	-0.03	0.05	0.22*	0.10*	0.23*	-0.19*	0.03	0.48*		0.25	0.08	-0.11	-0.01
	(0.04)	(0.03)	(0.03)	(0.04)	(0.03)	(0.10)	(0.04)	(0.04)	(0.04)	(0.05)	(0.07)	(0.04)	(0.03)		(0.17)	(0.22)	(0.47)	(0.36)
Mo	0.11*	0.20*	0.26*	0.07	0.23*	-0.00	0.14*	0.29*	0.25*	0.37*	0.14*	0.04	0.23*	0.21*		-0.12	-0.47	-0.14
	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)	(0.10)	(0.04)	(0.04)	(0.04)	(0.05)	(0.07)	(0.04)	(0.04)	(0.04)		(0.22)	(0.44)	(0.34)
I	-0.06	0.01	-0.05	0.08	-0.02	0.01	0.03	-0.05	0.03	-0.05	-0.01	-0.17*	-0.04	-0.00	0.14*		-0.40	
	(0.05)	(0.06)	(0.06)	(0.06)	(0.06)	(0.17)	(0.07)	(0.06)	(0.06)	(0.12)	(0.09)	(0.06)	(0.06)	(0.06)	(0.06)		(0.50)	-
Hg	0.07	-0.05	-0.16*	-0.11	-0.14*	0.04	0.13*	-0.04		0.07	0.06	0.10	0.01	0.00	0.03	-0.22*		-0.93
	(0.06)	(0.06)	(0.06)	(0.06)	(0.06)	(0.15)	(0.06)	(0.05)	-	(0.08)	(0.11)	(0.05)	(0.06)	(0.06)	(0.06)	(0.09)		(0.95)
B_{12}	0.13	-0.00	0.04	0.07	0.06	-0.01		0.05	0.03	0.19			0.02	0.16*	0.08		-0.10	
	(0.09)	(0.08)	(0.09)	(0.09)	(0.09)	(0.17)	-	(0.08)	(0.10)	(0.10)	-	-	(0.06)	(0.08)	(0.07)	-	(0.09)	

- Not estimable

* Significantly different from zero at $P<0.05$

Table 7. Correlations between element concentrations within serum. Additive genetic correlations are presented above the diagonal with phenotypic below. Corresponding standard errors are given in parenthesis

	Na	Mg	P	K	Ca	V	Mn	Fe	Co	Ni	Cu	Zn	Se	Mo	Cd	Hg	Pb
Na		0.60*		0.58*	0.57	0.24	0.50	0.45	0.27	0.40	-0.15	0.18	0.59	0.14	0.67		0.98*
		(0.37)	-	(0.24)	(0.56)	(0.29)	(0.66)	(0.40)	(0.39)	(0.35)	(0.34)	(0.36)	(0.337)	(0.33)	(0.35)	-	(0.07)
Mg	0.68*		0.43*	0.53*	0.49*	0.28	0.01	0.77*	-0.08	0.14	0.19	0.51*	-0.26	0.36	-0.18	0.05	-0.01
	(0.05)		(0.16)	(0.17)	(0.20)	(0.19)	(0.49)	(0.17)	(0.29)	(0.22)	(0.20)	(0.17)	(0.31)	(0.20)	(0.25)	(0.21)	(0.19)
P		0.68*		0.66*	0.67*	0.42*	-0.38	0.32	-0.05	-0.01	0.01	0.03	0.32	-0.02	0.03	-0.12	0.00
	-	(0.03)		(0.11)	(0.11)	(0.15)	(1.04)	(0.24)	(0.24)	(0.19)	(0.18)	(0.21)	(0.18)	(0.20)	(0.22)	(0.18)	(0.16)
K	0.71*	0.64*	0.71*		0.63*	0.45*	-0.22	0.39	0.05	0.10	0.17	0.23	0.20	0.55*	0.42	-0.20	0.05
	(0.05)	(0.03)	(0.02)		(0.14)	(0.16)	(0.49)	(0.25)	(0.25)	(0.20)	(0.19)	(0.20)	(0.24)	(0.18)	(0.21)	(0.18)	(0.17)
Ca	0.63*	0.70*	0.82*	0.77*		0.53*	-0.21	0.49	-0.06	0.07	0.26	0.34	0.08	0.48*	0.59*	-0.22	0.09
	(0.06)	(0.02)	(0.02)	(0.02)		(0.17)	(0.70)	(0.25)	(0.30)	(0.24)	(0.19)	(0.17)	(0.31)	(0.22)	(0.25)	(0.22)	(0.21)
V	0.09	0.37*	0.46*	0.42*	0.43*		0.63*	0.18	-0.05	0.31	0.03	0.21	0.21	0.57*	0.71*	-0.44*	-0.09
	(0.25)	(0.04)	(0.04)	(0.04)	(0.04)		(0.29)	(0.24)	(0.23)	(0.18)	(0.18)	(0.18)	(0.21)	(0.14)	(0.14)	(0.14)	(0.15)
Mn	0.34*	0.26*	0.30*	0.23*	0.26*	0.35*		0.02	0.23	-0.15	-0.03	0.50	-0.17	-0.20	0.28	0.08	-0.40
	(0.17)	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)		(0.60)	(0.49)	(0.45)	(0.43)	(0.43)	(0.58)	(0.48)	(0.44)	(0.39)	(0.44)
Fe	0.23	0.61*	0.55*	0.42*	0.55*	0.34*	0.29*		0.11	-0.21	0.17	0.43	-0.04	0.37	-0.52	-0.15	-0.12
	(0.15)	(0.03)	(0.03)	(0.04)	(0.03)	(0.04)	(0.04)		(0.34)	(0.27)	(0.25)	(0.24)	(0.34)	(0.24)	(0.30)	(0.25)	(0.24)
Co	0.33	0.57*	0.59*	0.45*	0.47*	0.32*	0.31*	0.54*		0.43	0.08	-0.16	0.10	-0.47	-0.26	-0.67*	-0.20
	(0.19)	(0.03)	(0.03)	(0.04)	(0.04)	(0.05)	(0.04)	(0.03)		(0.24)	(0.22)	(0.30)	(0.29)	(0.28)	(0.28)	(0.22)	(0.20)
Ni	-0.33	-0.01	-0.02	-0.03	0.02	0.07	0.10	0.01	0.05		0.11	0.10	0.41	0.21	0.22	-0.35*	0.29
	(0.50)	(0.06)	(0.06)	(0.06)	(0.06)	(0.07)	(0.06)	(0.06)	(0.06)		(0.18)	(0.21)	(0.25)	(0.21)	(0.21)	(0.14)	(0.16)
Cu	0.37*	0.61*	0.53*	0.56*	0.67*	0.35*	0.26*	0.47*	0.49*	0.06		0.22	0.11	0.19	-0.07	-0.30	-0.01
	(0.10)	(0.03)	(0.03)	(0.03)	(0.03)	(0.05)	(0.04)	(0.04)	(0.04)	(0.06)		(0.18)	(0.23)	(0.19)	(0.21)	(0.18)	(0.16)
Zn	-0.28	0.70*	0.62*	0.54*	0.70*	0.39*	0.34*	0.61*	0.55*	0.06	0.59*		-0.07	0.21	-0.04	-0.15	0.51*
	(0.19)	(0.02)	(0.03)	(0.03)	(0.03)	(0.04)	(0.04)	(0.03)	(0.04)	(0.06)	(0.03)		(0.24)	(0.22)	(0.24)	(0.19)	(0.17)
Se	0.71*	0.58*	0.73*	0.60*	0.75*	0.37*	0.24*	0.48*	0.58*	-0.01	0.66*	0.59*		0.31	-0.20	-0.19	0.32
	(0.05)	(0.03)	(0.02)	(0.03)	(0.02)	(0.04)	(0.04)	(0.04)	(0.03)	(0.06)	(0.03)	(0.03)		(0.26)	(0.28)	(0.24)	(0.21)
Mo	0.32*	0.33*	0.18*	0.23*	0.16*	0.35*	0.10	0.32*	0.24*	0.05	0.31*	0.30*	0.14*		0.10	-0.21	0.49*
	(0.10)	(0.04)	(0.05)	(0.04)	(0.05)	(0.05)	(0.05)	(0.04)	(0.05)	(0.07)	(0.04)	(0.05)	(0.05)		(0.22)	(0.19)	(0.15)
Cd	0.02	0.14*	0.09*	0.16*	0.14*	0.34*	0.16*	0.08	0.13*	0.11	0.10*	0.15*	0.05	0.23*		-0.23	-0.24
	(0.16)	(0.00)	(0.05)	(0.04)	(0.05)	(0.04)	(0.04)	(0.05)	(0.05)	(0.07)	(0.05)	(0.05)	(0.05)	(0.05)		(0.17)	(0.18)
Hg		0.05	0.11	0.04	-0.01	-0.20*	0.03	0.01	-0.17*	-0.19*	-0.05	-0.04	-0.01	-0.10	-0.10		-0.11
	-	(0.08)	(0.08)	(0.07)	(0.07)	(0.08)	(0.07)	(0.07)	(0.07)	(0.09)	(0.08)	(0.08)	(0.07)	(0.09)	(0.07)		(0.13)
Pb	0.86*	0.11*	0.21*	0.13*	0.16*	0.13*	0.10*	0.14*	0.11*	0.19*	0.10*	0.24*	0.19*	0.26*	-0.01	-0.04	
	(0.03)	(0.05)	(0.05)	(0.05)	(0.04)	(0.05)	(0.05)	(0.05)	(0.05)	(0.06)	(0.05)	(0.05)	(0.04)	(0.05)	(0.05)	(0.08)	

- Not estimable

* Significantly different from zero at *P<0.05*

611 **Table 8.** Additive genetic correlations (*r*) between milk and serum element concentrations
612 with corresponding standard errors (S.E.) and P-values (*P*). Only correlations significantly
613 different from zero (at *P<0.05*) results are presented

Milk	**Serum**	***r* (S.E.)**	***P***
Sodium (Na, mg/L) [q]	Calcium (Ca, mg/L) [q]	0.56 (0.275)	0.049
Sodium (Na, mg/L) [q]	Nickel (Ni, µg/L) [t]	-0.62 (0.244)	0.017
Magnesium (Mg, mg/L) [q]	Iron (Fe, µg/L) [t]	0.65 (0.266)	0.021
Phosphorus (P, mg/L) [q]	Magnesium (Mg, mg/L) [q]	0.49 (0.215)	0.029
Potassium (K, mg/L) [q]	Cadmium (Cd, µg/L) [h]	0.43 (0.194)	0.035
Potassium (K, mg/L) [q]	Cobalt (Co, µg/L) [t]	0.48 (0.222)	0.039
Potassium (K, mg/L) [q]	Magnesium (Mg, mg/L) [q]	0.53 (0.189)	0.008
Potassium (K, mg/L) [q]	Molybdenum (Mo, µg/L) [t]	0.45 (0.182)	0.020
Potassium (K, mg/L) [q]	Potassium (K, mg/L) [q]	0.45 (0.192)	0.025
Calcium (Ca, mg/L) [q]	Magnesium (Mg, mg/L) [q]	0.54 (0.210)	0.014
Vanadium (V, µg/L) [t]	Nickel (Ni, µg/L) [t]	-0.98 (0.354)	0.008
Manganese (Mn, µg/L) [t]	Cadmium (Cd, µg/L) [h]	0.77 (0.219)	<0.001
Manganese (Mn, µg/L) [t]	Magnesium (Mg, mg/L) [q]	0.57 (0.247)	0.028
Cobalt (Co, µg/L) [t]	Nickel (Ni, µg/L) [t]	-0.86 (0.319)	0.011
Selenium (Se, µg/L) [t]	Cadmium (Cd, µg/L) [h]	-0.46 (0.210)	0.036
Molybdenum (Mo, µg/L) [t]	Iron (Fe, µg/L) [t]	0.68 (0.302)	0.031

614 q Quantity element
615 t Trace element
616 h Heavy metal
617

Table 9. Phenotypic correlations (*r*) between milk and serum elements and vitamin B_{12} with corresponding standard errors (S.E.) and P-values (*P*). Only correlations significantly different from zero (at *P<0.05*) results are presented

Milk	Serum	*r* (S.E.)	*P*
Sodium (Na, mg/L) [q]	Cadmium (Cd, µg/L) [h]	0.39 (0.070)	<0.001
Sodium (Na, mg/L) [q]	Lead (Pb, µg/L) [h]	0.36 (0.074)	<0.001
Sodium (Na, mg/L) [q]	Sodium (Na, mg/L) [q]	-0.79 (0.175)	<0.001
Magnesium (Mg, mg/L) [q]	Iron (Fe, µg/L) [t]	0.19 (0.064)	0.004
Magnesium (Mg, mg/L) [q]	Sodium (Na, mg/L) [q]	0.49 (0.208)	0.024
Phosphorus (P, mg/L) [q]	Cadmium (Cd, µg/L) [h]	0.32 (0.072)	<0.001
Phosphorus (P, mg/L) [q]	Calcium (Ca, mg/L) [q]	0.15 (0.071)	0.043
Phosphorus (P, mg/L) [q]	Iron (Fe, µg/L) [t]	0.15 (0.069)	0.037
Phosphorus (P, mg/L) [q]	Magnesium (Mg, mg/L) [q]	0.15 (0.070)	0.037
Potassium (K, mg/L) [q]	Cadmium (Cd, µg/L) [h]	0.30 (0.074)	<0.001
Potassium (K, mg/L) [q]	Calcium (Ca, mg/L) [q]	0.18 (0.069)	0.014
Potassium (K, mg/L) [q]	Cobalt (Co, µg/L) [t]	0.21 (0.066)	0.002
Potassium (K, mg/L) [q]	Iron (Fe, µg/L) [t]	0.15 (0.068)	0.037
Potassium (K, mg/L) [q]	Magnesium (Mg, mg/L) [q]	0.20 (0.067)	0.005
Potassium (K, mg/L) [q]	Molybdenum (Mo, µg/L) [t]	0.18 (0.076)	0.027
Potassium (K, mg/L) [q]	Phosphorus (P, mg/L) [q]	0.16 (0.064)	0.019
Potassium (K, mg/L) [q]	Potassium (K, mg/L) [q]	0.19 (0.067)	0.006
Potassium (K, mg/L) [q]	Sodium (Na, mg/L) [q]	0.64 (0.173)	<0.001
Calcium (Ca, mg/L) [q]	Cadmium (Cd, µg/L) [h]	0.22 (0.078)	0.006
Calcium (Ca, mg/L) [q]	Calcium (Ca, mg/L) [q]	0.17 (0.070)	0.019
Calcium (Ca, mg/L) [q]	Iron (Fe, µg/L) [t]	0.16 (0.068)	0.024
Calcium (Ca, mg/L) [q]	Magnesium (Mg, mg/L) [q]	0.14 (0.069)	0.047
Calcium (Ca, mg/L) [q]	Sodium (Na, mg/L) [q]	0.81 (0.118)	<0.001
Vanadium (V, µg/L) [t]	Lead (Pb, µg/L) [h]	0.53 (0.107)	<0.001
Chromium (Cr, µg/L) [t]	Selenium (Se, µg/L) [t]	-0.16 (0.070)	0.029
Iron (Fe, µg/L) [t]	Cadmium (Cd, µg/L) [h]	-0.28 (0.086)	0.002
Iron (Fe, µg/L) [t]	Lead (Pb, µg/L) [h]	-0.62 (0.046)	<0.001
Cobalt (Co, µg/L) [t]	Cadmium (Cd, µg/L) [h]	0.56 (0.063)	<0.001
Zinc (Zn, µg/L) [t]	Cobalt (Co, µg/L) [t]	-0.15 (0.066)	0.033
Zinc (Zn, µg/L) [t]	Sodium (Na, mg/L) [q]	0.74 (0.142)	<0.001
Selenium (Se, µg/L) [t]	Calcium (Ca, mg/L) [q]	0.15 (0.068)	0.037
Molybdenum (Mo, µg/L) [t]	Cadmium (Cd, µg/L) [h]	0.22 (0.072)	0.004
Molybdenum (Mo, µg/L) [t]	Iron (Fe, µg/L) [t]	0.17 (0.063)	0.011
Molybdenum (Mo, µg/L) [t]	Molybdenum (Mo, µg/L) [t]	0.19 (0.070)	0.009
Mercury (Hg, µg/L) [h]	Copper (Cu, µg/L) [t]	-0.23 (0.074)	0.003
Mercury (Hg, µg/L) [h]	Molybdenum (Mo, µg/L) [t]	-0.20 (0.089)	0.034
Vitamin B_{12} (B_{12}, µg/L)	Cadmium (Cd, µg/L) [h]	0.21 (0.092)	0.028
Vitamin B_{12} (B_{12}, µg/L)	Nickel (Ni, µg/L) [t]	-0.38 (0.096)	<0.001

q Quantity element
t Trace element
h Heavy metal